The Functional Role of Cardiac Troponin I Tyrosine Phosphorylation

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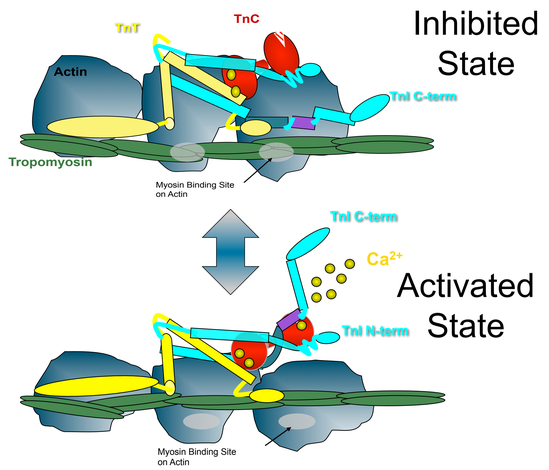
Introduction

It has been recently shown that phosphorylation of the human cardiac troponin inhibitory subunit (TnI) at Tyr-26 is decreased in heart failure with functional effects to decrease thin filament calcium sensitivity and accelerate calcium dissociation. This discovery has allowed us to hypothesize that the phosphorylation of additional Tyr sites in the TnI subunit, including Tyr-29, Tyr-112 (and Tyr-134 found mouse cardiac muscle), will also exhibit functional effects. Kinase prediction calculation suggests that these additional sites are more likely to be phosphorylated than Tyr-26. To test the regulatory effects of TnI Tyr-phosphorylation, we generated, expressed and purified recombinant cardiac TnI containing phosphomimetic substitutions (Tyr to Glu). Purified proteins were used to form the troponin complex and functional effects determined by measuring calcium binding to troponin C (TnC) in reconstituted thin filaments.

Troponin Regulation of Contraction

Adapted From: Kass, D. A. and R.J. Solaro, Circulation 2006.



Force can be regulated by altering the calcium binding properties of TnC through post-translational modification of the Tn subunits.

TnI Tyr phosphorylation

Phosphorylation of TnI Tyr-26 is decrease in the human heart failure *(Zhang et al., 2012)*.

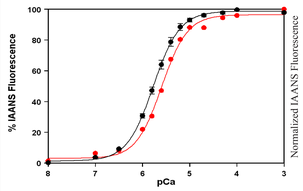


Fig 1A. Phosphorylation of Tyr-26 decreases calcium sensitivity. Normalized steady-state calcium binding in thin filaments reconstituted with either Tn containing cTnI Wt (**Black; n=4**), or Tyr26E (**Red; n=3**) as the change in IAANS fluorescence upon TnC-Ca^{2+} binding. *; $p < 0.05$. (Salhi, H., *et al.*, 2014)

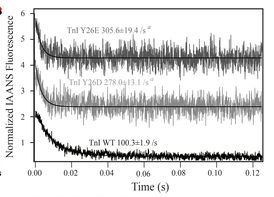


Fig 1B. TnI Tyr-26 pseudo-phosphorylation accelerates Ca^{2+} dissociation from TnC on the thin filament. Representative change in IAANS fluorescence of stopped-flow Ca^{2+} dissociation from thin filaments containing non-phosphorylated WT TnI (*TnI WT, black line, n=12*), or pseudo-phosphorylated TnI Tyr-26 (*TnI Y26D, light grey line, n=18* and *TnI Y26E, dark grey line, n=18*). Traces are normalized and staggered for clarity. *a*, significantly different from TnI WT $p<0.001$ (Salhi, H., *et al.*, 2014)

HcTnI Tyr-26 phosphorylation decreases calcium sensitivity and accelerates calcium dissociation.

In addition to Human Tyr-26, Mouse TnI contains an additional Tyr at residue 134

Hypothesis and Aims

Hypothesis:

TnI Tyrosine residue phosphorylation modulates calcium regulated thin filament activation to affect the production of cardiac force.

Three Aims/Experiments:

1. **Mouse versus Human WT TnI**
   (McTnI WT +Y26E / HcTnI WT + Y26E)
2. **All TnI Tyr residue phosphorylation**
   (McTnI Y26/29/112/134E / HcTnI Y26/29/112E)
3. **TnI Tyr-134 phosphorylation**
   (McTnI Y134E / HcTnI F134E)

Methods:

These aims will be tested by measuring calcium binding and dissociation in reconstituted thin filaments.

Methods

① DNA mutagenesis
② Transformation/expression in Rosetta cells
③ Purification via column chromatography

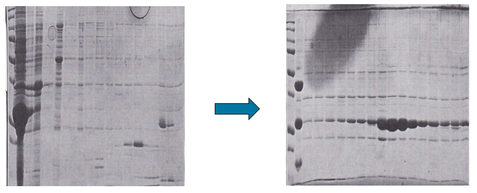
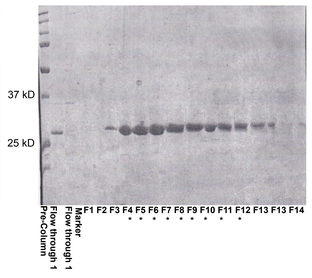


Fig. 2A. SDS-PAGE gel of McTnI Y26E after purification via anion-exchange affinity chromatography. Fractions containing TnI protein were selected and pooled for TnC-affinity column chromatography.

McTnI Y26E

Fig. 2B. SDS-PAGE gel of McTnI Y26E after purification via TnC affinity column chromatography. Pure McTnI containing fractions (*).

Pure McTnI Y26E containing fractions (*) determined by SDS-PAGE electrophoresis and pooled for Tn complex formation



Mouse versus Human TnI

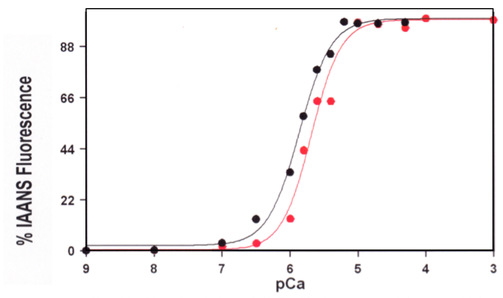


Fig. 3A. Phosphorylation of HcTyr-26 decreases calcium sensitivity. Representative reconstituted thin filament calcium binding curves demonstrate decreased calcium sensitivity of HcTn Y26E (red) compared to HcTn Wt (black).

Results are consistent with previous study.

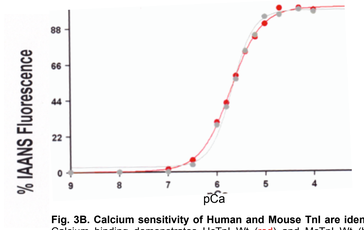


Fig. 3B. Calcium sensitivity of Human and Mouse TnI are identical. Calcium binding demonstrates HcTnI Wt (red) and McTnI Wt (black) exhibit identical calcium sensitivity in reconstituted thin filaments.

Phosphorylation at HcTnI Tyr-26 decreases calcium sensitivity. WT Human and Mouse TnI exhibit similar calcium sensitivity.

Mouse TnI Y26E

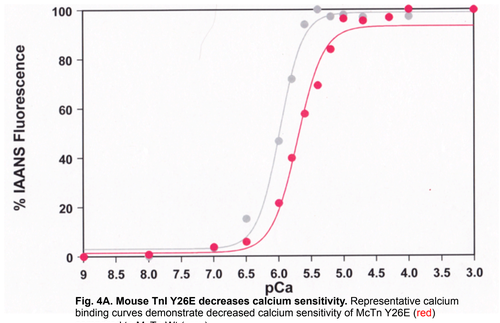


Fig. 4A. Mouse TnI Y26E decreases calcium sensitivity. Representative calcium binding curves demonstrate decreased calcium sensitivity of McTn Y26E (red) compared to McTn Wt (grey).

McTnI Y26E phosphorylation decreases calcium sensitivity similar to that of HcTnI Y26E.

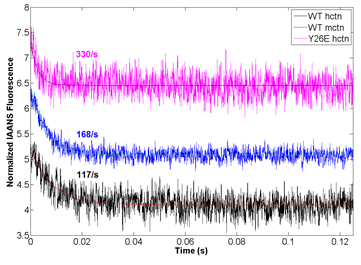


Fig 4B. Mouse TnI Y26E accelerated calcium dissociation. Representative change in IAANS fluorescence of stopped-flow Ca^{2+} dissociation from thin filaments containing non-phosphorylated WT HcTnI (*TnI WT, blue line*), *non-phosphorylated WT McTnI* (*TnI WT, black line*), Tyr-26 pseudo-phosphorylated TnI (*TnI HcY26E, pink line*). Traces are normalized and staggered for clarity.

Summary

- Pseudophosphorylation of cardiac TnI at Mouse Tyr-26 decreases calcium sensitivity similarly to that of Human Tyr-26.

Future Experiments:

- Aim 2: Looking at the effects of phosphorylation at all tyrosine sites in human and mouse cardiac Tn
  - Have purified proteins McTnI Y26/29/112/134E as well as HcTnI Y26/29/112E for experimentation
- Aim 3: Looking at effect of sole phosphorylation at site 134
  - Have purified proteins McTnI Y134E and HcTnI 134E for further experimentation

We are beginning to study the functional methods of the various phosphorylations in regards to thin filament calcium binding properties by running calcium binding experiments on TnC which could possibly show a contribution similar to Tyr-26, showing altered calcium sensitivity